CARBON DIOXIDE UPTAKE OF LEAVES AND PH VALUE OF THE CELL SAP

by

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Introduction

In 1963 the Council of Mutual Economic Aid has coordinated the joint scientific program of the countries within its compass. This organizatory work has tightened the cooperation between the research workers of the Polish and the Hungarian People's Republic. Photosynthesis is a range of problems which Hungarian research workers have not much dealt with (cf. 1959 study of Tadeusz Wojtaszek, Poland); within the framework of cooperation we try to remedy this deficiency.

Penetration of atmospherical carbon dioxide into the leaf cells represents the initial process of the photosynthetic activity of plants; only as a consequence thereof can the encounter with proper carbon dioxide acceptors take place. In reality, however, research on photosynthesis begins to deal with the problem at this second step (e. g. Vejlby 1960), mainly with the discussed question of 2 C- and 5 C-atomic acceptors.

One of the most ignored basic problems of carbon dioxide fixation – either photosynthetic or going on for a while in darkness – is, how can the atmospherical carbon dioxid as a very mild anhydro acid, penetrate into the cells of plants despite the acidity of cell sap? Acidity can be comparatively very high; for instance, Ruhland and Wetzel (1926) have measured pH values of 1.3 to 1.6 for the leaf of *Begonia semperflorens*, while Rogers and Shive (1932) have measured pH value of 1.9 to 2.2 and 2.5 to 2.7 in the case of *Oxalis repens* and *Rumex acetosella*, respectively. These data are sufficiently emphasizing the problem, since the above-mentioned pH values, as compared with the very low acidity of atmospherical CO_2 and of H_2CO_3, might appear as an extraordinary obstacle to solution. For further information, reference is made to the work of J. Small (1954).

Although the protoplasm is not a homogeneous but a microheterogeneous system containing basic groups too, problems concerning dissolution cannot be avoided. Of whatever nature the CO_2-acceptor assumed, its complete isolation from cell sap is hardly conceivable. It must be also taken into account, that

CO_2, penetrating into intracellular ducts of the leaf, cannot get inside the cells unless it penetrates the hydrate cover of cell walls bordering the ducts. According to hydrature, this cover is presumably of varying dimension and is not likely to contain any special CO_2-acceptors.

It is obvious that the very first step of CO_2 cannot be anything else than dissolution; even the pH value of the inside environment happens to be inferior to that of carbon dioxide. Since acids stronger than carbon dioxide are releasing CO_2 gas from carbonic salts, an erroneous opinion has gained ground according to which CO_2 is insoluble in any medium of stronger acidity. This is how the first question of the plants' CO_2 uptake could have been thrust into the background and it is this issue the approximative examination of which we have attempted to perform in the present paper.

Material and Method

The experiments have been conducted at room temperature and at 30 – 32° C, corresponding to summer heat, with carbonic acid factory gas, as well as with CO_2 gas released from $NaHCO_3$ and controlled for chemical purity. On the prevailing atmospherical pressure, gas was put in contact over a large surface with the test fluids:

1. with vegetable saps purified by means of centrifuging,
2. with the aqueous solution of mild acids,
3. with the aqueous solution of strong acids.

The absorption of gas, making contact with the above acidic solutions, was volumetrically measured by means of our special gas-pipette (F r e n y ó, 1963) and of a simple eudiometer, while the pH value of the solutions was electrometrically determined by means of a „METROHM"-type apparatus.

The essence of the gas-pipette method is as follows with the aid of a threaded nozzle, gas sample and absorbent fluid are brought into the measuring capillary and the respective volumes become established; then the gas-sample is intensely mixed with the fluid by twisting the nozzle. The per cent by volume of gas dissolved under the given conditions in the given amount of fluid is developed from the decrease of the original volume. The principle of our method resembles unintentionally to that coming into display in the gas-pipette of M a k a s e v (1959).

Eudiometric measurement was made by bringing CO_2 gas of previously measured volume into a scaled tube filled with the test fluid and immersed into mercury with its open end. After intensive contact with the fluid, the remaining volume of the gas-column was again established.

Experimental results

Since our measurements about CO_2 absorption of vegetable saps were designed for preliminary information only, we are not going to present the data obtained in full detail. As a rule, we have found that – provided the vegetable saps did not contain uncommonly high amounts of hydrocarbonate – they absorbed CO_2 gas in a readily traceable way. For instance, the sap obtained from the leaf of *Sambucus nigra* (pH 6) has dissolved, at a temperature

of 28° C and within 2 to 5 minutes, the intensely contacting gas at a rate of 10 cu. mm/1 ml, while the sap of *Tradescantia albiflora* (pH 3.5) absorbed 40 cu. mm of CO_2 gas per 1 ml of sap within a few minutes.

Among the organic acids we have, first of all, examined some members belonging to the Szent-Györgyi – Krebs cycle in order to find out the amount of CO_2 gas absorbed by their aqueous solution. Dependent on their own solubility, these acids had to be separated into two groups. As a rule, 0.1% solutions were prepared out of fairly water-soluble acids; from less soluble acids we filled as much into distilled water as could be dissolved at the prevailing environmental temperature of 30° C by water. Thus we have made saturated solutions out of hardly soluble acids without numerically determining their concentration.

Among readily soluble acids of the Szent-Györgyi–Krebs cycle, citric acid was used as a dilution series. The results obtained are shown in Table 1.

Table 1.

Dissolution of CO_2 in Some Acids of the Krebs Cycle
(at 30°C, within 2 minutes)

Name of acid	Concentration of aqueous sol.	pH	Amount of dissolved CO_2 (Cu. mm.) related to 1 ml of solvent. (Not from infinite quantity)
Citric acid	25,00%	1,65	aproximately 108
,, ,,	12,50%	1,84	,, 134
,, ,,	6,25%	2,09	,, 163
,, ,,	3,12%	2,20	,, 210
Malic acid	0,10%	1,50	,, 95
Aconite acid	0,10%	1,10	,, 205
Succinic acid	saturated	1,50	,, 175
Fumar acid	saturated	1,80	,, 103

Other organic acids were also examined from this point of view, with saturated solutions prepared out of hardly water-soluble types. The data are shown in Table 2.

Table 2.

Dissolution of CO_2 in Different Organic Acids
(30°C, 2 min.)

Name of acid	Concentration of aqueous sol.	pH	Amount of dissolved CO_2 (Cu. mm.) related to 1 ml of solvent. (Not from infinite quantity)
Ascorbic acid	0,1%	1,9	aproximately 225
Tartaric acid	0,1%	1,5	,, 175
Malonic acid	0,1%	0,9	,, 195
Maleic acid	0,1%	0,7	,, 230
Amino acetic acid (glycine)	0,1%	5,5	,, 310
Aspartic acid	saturated	2,3	,, 140
Glutamic acid	saturated	2,7	,, 235

In addition to those enumerated in Tables 1. and 2., other organic acids were also submitted to dissolution tests. However, since these tests were not systematical, the resulting data are not included in the Tables. The behaviour of oxalic acid was most remarkable: 1 ml 0.1 n oxalic acid completely absorbed 20 cu. mm of CO_2 gas within 1 minute.

Among the strong acids, we examined hydrochloric and sulfuric acid to establish the absorbability of their respective aqueous solutions of various concentration. Performed at the prevailing temperature of 31°C, the reference tests have shown that CO_2 gas is most readily absorbed by these strong and well dissociated anorganic acids: 1 ml of their 10% solution actually absorbed 115 – 140 cu. mm of gas within a few minutes. The precise investigation, performed at a temperature of 25° C, has shown that 1 ml of 10% H_2SO_4 is able to absorb as much as 710 – 720 cu. mm of CO_2, if it is in contact with an infinite amount of gas. Absorption is increased by the decrease of concentration: 1 ml of 5% H_2SO_4 is saturated by the uptake of 740 – 750 cu. mm of CO_2.

Discussion

Relying upon our experimental data we are of the opinion that the first step of CO_2 fixation by plant cells is being needlessly evaded by scientific interpretation. Although CO_2 is an anhydro acid so mild that the pH value of its saturated aqueous solution is only about 4.6, it still can be absorbed by far more acidic saps of plant cells. Our investigations have shown that the CO_2-absorption of the strongest anorganic acids is not much lower than the CO_2-solution of clear water. According to the Table of Seidell – Linke (1951), 1 ml of H_2O is able to dissolve 0.823 ml of CO_2 at normal atmospherical pressure and at a temperature of 25° C. Our data obtained with sulfuric and hydrochloric acid are only 10% lower. Encouraged by this fact we assume that, first of all, CO_2 absorbed by the plant from its environment is dissolved in the saps of the plant, while the role of specific acceptors is only subsequent. We have seen that all of the examined organic acids and the vegetable sap itself dissolve the CO_2 gas to an adequate extent to permit the initial step of uptake to be taken.

However, the mass action law is highly operative under the conditions applied in our experiments, on one side, and the natural conditions are, on the other. It is obvious that the dissolution of 0,03% atmospherical CO_2 is not as intensive as it would be at the same pressure but in a high concentration. We have to deal here with a case of Henry's law; dependent on the concentration, more or less gas molecules collide with the liquid phase and get over from the gas space to the liquid space. Under natural conditions, in some lifeless system, e. g. between water and atmosphere, equilibrium takes a comparatively long time to be established and it is but natural, that, out of the low-concentration CO_2 stock, only a small amount is constantly in solution. The general form of the equation, as known from chemical mechanics and including the mass action law, i. e.

$$n_1 \cdot A + n_2 \cdot B + n_3 \cdot C + \ldots \rightleftharpoons m_1 \cdot X + m_2 \cdot Y + m_3 \cdot Z \ldots$$

will be valid in any system; in this way, proper CO_2 supply cannot be explained as yet. However, the system between the intercellular space and the cells is not a static one. Cyclically regenerating in the cells, the photosynthetic CO_2-acceptors permit a constant unidirectional shift of the gas equilibrium and, to its expense, an average photosynthetic performance of 20 mg CO_2/100 sq. cm/h to be accomplished.

Our observations concerning the dissolution of CO_2 can account not only for the initial part of CO_2 uptake related to photosynthesis, but they may be of practical importance chiefly in the field of gas analysis. Probably due to the pioneering works of B u n s e n, it has become a current practice to acidify fluids in order to prevent them from absorbing CO_2 gas. Instructions of this kind can be found in several methodics, in fact, our own previous works are not exempt from this error; e. g. in our examinations on the composition of intercellular gas mixture (F r e n y ó 1963), the data obtained for the concentration of CO_2 must be increased by means of a correction factor.

Summary

The present paper supplies arguments to account for the first step of the plants' CO_2 uptake; this initial phase does not require any special CO_2-acceptor, but can be interpreted in terms of dissolution. The acidic reaction of cell sap does not inhibit at all the absorption of the much milder CO_2. Of course, a continuous uptake is possible if, prior to the saturation of the absorber medium, CO_2 is being used up in photosynthesis.

In the course of the experiments it has turned out that, in principle, it is a wrong practice to try to inhibit the dissolution of CO_2 by acidifying the medium. Consequently, certain procedures of gas-analytics must be reconsidered.

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Résumé

L'étude fournit des arguments pour expliquer le premier pas de la capture de CO_2, effectuée par les plantes. Sans exigeant un accepteur spécial de CO_2, cette phase initiale s'interprête en termes de la dissolution. La réaction acide du liquide cellulaire n'empêche en rien l'absorption de CO_2 qui est pourtant bien moins acide. Évidemment, une capture continue n'est possible que si CO_2est utilisé dans la photosynthèse avant la saturation du médium absorbant.

On a établi, au cours des expériences qu'il est, en principe, incorrect de tâcher d'empêcher la dissolution de CO_2 en acidifiant le médium. Donc, certains procédés techniques de l'analyse de gas doivent être révisés.

РЕЗЮМЕ

В статьи приводятся аргументы для объяснения принимания CO_2 растениями в первом шаге этого процесса; данная исходная часть не требует специального акцепторя для CO_2, но она можебыть объяснена при помощи растворения. Кислая химическая реакция клеточной влаги совсем не мещает абсорбированию намного меньше кислого CO_2. Разумеется, непрерывное принимание становится возможным путем израсходования CO_2 в фотосинтезе до насыщения абсорбирующей среды.

В процессе исследований выяснилось, что распространенная практика, при которой стремятся к препятствованию растворения CO_2 путем окисления среды, является принципиально ошибочной. В смысле вышесказанного следует проверить известные газоаналитические методы.

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